REMARKS

This is meant to be a complete response to the Office Action mailed July 22, 2008. In the Office Action, the Examiner rejected Applicants' claims 31-37, 42, 45-51, 60 and 61 under 35 U.S.C. 112, first paragraph (written description requirement), and claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 112, first paragraph (written description requirement). Also in the Office Action, the Examiner rejected claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 103(a) as being unpatentable over US 5,482,841 in view of US 5,292,641, Prilliman et al., DiBrino et al., and Zemmour et al. Further, the Examiner rejected claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 103(a) as being unpatentable over US 5,482,841 in view of US 5,292,641, US 2002/0197672, Prilliman et al., and DiBrino et al.

Amendments to the Claims

Claim 31 has been amended herein to recite that the mammalian cell line utilized in the method recited therein "expresses multiple surface-bound native Class I endogenous MHC molecules". Support for said amendment can be found in Paragraphs [0055], [0059] and [0065] of the published application of the subject application, as well as paragraph [0011] of parent application US Serial No. 09/974,366, which was expressly incorporated by reference in the subject application. Therefore, Applicants respectfully submit that the amendment to claim 31 does not constitute new matter and fully complies with the written description requirement of 35 U.S.C. 112.

Applicants' Response to the First Written Description Rejection

In the Office Action, the Examiner rejected Applicants' claims 31-37, 42, 45-51, 60 and 61 under 35 U.S.C. 112, first paragraph (written description requirement). In support thereof, the Examiner stated that:

> The amendatory material not supported by the disclosure as originally filed is as follows: "at least one MHC trimolecular complex linked thereto" recited in instant base claim 31, "at least one MHC trimolecular complex" recited in claims 35 and in claim 61.
> Applicant did not point to support in the disclosure for the claim amendments.

In response thereto, Applicants submit that there is clear support for the term "at least one MHC trimolecular complex" in the originally filed application. Paragraph [0087] of the subject application clearly states that: "an intact sHLA molecule is a complex consisting of HC, β2m and a peptide".

Further, Paragraph [0015] from parent application US Serial No. 10/022,066, which was specifically incorporated by reference in the subject application (see paragraph [0002] thereof), states that:

> Class I molecules primarily associate with peptide fragments, thus forming **α-chain/β2m/peptide trimolecular complexes**, via an endogenous processing pathway during their assembly (Germain 1994; Heemels and Ploegh 1995; Lehner and Cresswell 1996; York and Rock 1996; Pamer and Cresswell 1998); in fact, the very binding of peptides is essential for the stabilization and expression of these molecules (Ljunggren et al. 1990; Townsend et al. 1990; Elliott 1991).

Therefore, Applicants respectfully submit that claims 31-37, 42, 45-51, 60 and 61 fully comply with the written description requirement of 35 U.S.C. 112, first paragraph. Applicants respectfully request reconsideration and withdrawal of the rejection.

Applicants' Response to the Second Written Description Rejection

In the Office Action, the Examiner rejected Applicants' claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 112, first paragraph (written description requirement). In support thereof, the Examiner stated that:

> The amendatory material (in the prior amendment filed 8/20/07) not supported by the disclosure as originally filed is as follows: "wherein the mRNA encodes at least one MHC heavy chain allele" recited in claim 31.
> Applicant did not point to support in the disclosure for the claim amendment.

In response thereto, Applicants respectfully submit that there is clear support for the phrase "wherein the mRNA encodes at least one MHC heavy chain allele" in the originally filed application. Paragraph [0013] refers to "HLA allele mRNA", while Paragraph [0015] states that: "[t]he functionally active, individual soluble HLA molecules may be produced by several methods, including but not limited to the following. In one embodiment, ***HLA allele mRNA*** from a source is isolated and reverse transcribed to obtain allelic cDNA" (emphasis added).

In addition, Figures 1 and 2, as well as their corresponding legends and detailed descriptions, clearly describe that the "mRNA encodes at least one MHC heavy chain allele", and that it is the product of the MHC heavy chain allele (i.e., the MHC heavy chain molecule) that is truncated by PCR amplification to remove the cytoplasmic and transmembrane domains of the MHC heavy chain molecule. For example, paragraph [0023] states that:

> FIG. 1 is a graphical representation of a Class I location and sHLA class I construction strategy. (A) Simple map of the human MHC region with the class I HLA-B, -C, and -A loci noted. Genetic distances are in kilobases. (B) The basic exon structure of HLA class I gene transcripts. Seven exons encode the ***class I heavy chain***. (C) ***PCR strategy for truncating the class I molecule*** so that it is secreted rather than surface bound. (emphasis added)

In addition, paragraph [0066] states that:

> HLA class I molecules are antigen-presenting glycoproteins expressed universally in nucleated cells. In humans, heavy chains are encoded at 3 loci (B, C, and A) within the MHC on the short arm of chromosome 6 (FIG. 1A). FIG. 1B illustrates each α-chain comprised of α1, α 2, and α 3 domains, as well as a transmembrane domain, which tethers the molecule to the cell surface and a short C-terminal cytoplasmic domain. In contrast, the light chain is encoded outside of the MHC (on chromosome 15 in humans) and bears no such anchoring domain; it instead associates noncovalently with the α3

domain of the heavy chain. FIG. 1C illustrates the approach for creating sHLA class I transcripts. The PCR primers truncate the class I heavy chain following exon 4, just before the transmembrane domain and cytoplasmic domains.

Therefore, Applicants respectfully submit that claims 31-37, 42, 45, 46, 48-51, 60 and 61 fully comply with the written description requirement of 35 U.S.C. 112, first paragraph. Applicants respectfully request reconsideration and withdrawal of the rejection.

Applicants' Response to the First 35 U.S.C. 103(a) Rejection

In the Office Action, the Examiner rejected Applicants' claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 103(a) as being unpatentable over US 5,482,841 in view of US 5,292,641, Prilliman et al., DiBrino et al., and Zemmour et al. Applicants respectfully traverse the rejection for the reasons stated herein below.

The present invention, as recited in the claims, is a method for detecting the presence of anti-MHC antibodies in a sample. In the method, a pool of functionally active, recombinantly produced, truncated individual soluble MHC trimolecular complexes is obtained by the steps of isolating mRNA encoding at least one MHC heavy chain allele from a source, reverse transcribing the mRNA to obtain cDNA; identifying an individual MHC heavy chain allele in the cDNA; and PCR amplifying the individual MHC heavy chain allele in a locus-specific manner to produce a PCR product having the coding regions encoding cytoplasmic and transmembrane domains of the individual MHC heavy chain allele removed such that the PCR product encodes a truncated, soluble form of the individual MHC heavy chain molecule. The PCR product is then cloned into a mammalian expression vector, thereby forming a construct that encodes the individual soluble MHC heavy chain molecule, and the construct is transfected into a mammalian cell line that **expresses multiple surface-bound native Class I endogenous MHC molecules**. The mammalian cell line is then cultured under conditions which allow for expression of the recombinant individual soluble MHC heavy chain molecule from the construct, such conditions also allowing for endogenous loading of a peptide ligand into the antigen binding groove of each individual soluble MHC heavy chain molecule in the presence of

beta-2-microglobulin to form the individual soluble MHC trimolecular complexes prior to secretion of the individual soluble MHC trimolecular complexes from the cell, wherein each trimolecular complex comprises a recombinant, soluble MHC heavy chain allele, beta-2-microglobulin and endogenously loaded peptide and wherein each trimolecular complex of the pool of functionally active, recombinantly produced, truncated individual soluble MHC trimolecular complexes has the same recombinant, soluble MHC heavy chain allele. **The individual, soluble MHC trimolecular complexes are then purified substantially away from other proteins** and maintain the physical, functional and antigenic integrity of the native MHC trimolecular complex.

At least one soluble MHC trimolecular complex is then linked directly or indirectly to a substrate such that it retains the physical, functional and antigenic integrity of the native MHC trimolecular complex. A sample is then reacted with the substrate/MHC trimolecular complex and washed to remove unbound portions of the sample. The substrate/MHC trimolecular complex is then reacted with means for detecting anti-MHC antibodies, and it is determined that anti-MHC antibodies specific for the individual MHC molecule are present in the sample if the means for detecting anti-MHC antibodies is positive.

The fact that the Examiner had to combine teachings from **five** different references in the 35 U.S.C. 103(a) rejection demonstrates that a case of *prima facie* obviousness has not been established, and that the Examiner has impermissibly used "hindsight", based on the teachings provided in the subject application, to hunt through the prior art for the claimed elements and combine them as claimed, and such approach is "an illogical and inappropriate process by which to determine patentability" (*Sensonics, Inc. v. Aerosonic Corp.*, 81 F.3d 1566, 1570, 38 USPQ2d 1551, 1554 (Fed. Cir. 1996). The Examiner has taken into account knowledge that was NOT within the level of ordinary skill in the art at the time the claimed invention was made. The Examiner used knowledge gleaned only from the Applicants' disclosure, and thus the "hindsight reconstruction" was improper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). Thus, a case of *prima facie* obviousness has clearly not been established.

However, for the sake of expediting issuance of a patent from the subject application, Applicants respectfully submit that the presently claimed invention is not obvious over the **combination** of the teachings of all five references, as described in more detail herein below.

US 5,482,841 teaches a method of evaluating transplant acceptance **using membrane bound alloantigen that has been extracted from cells with a mild detergent** (see Column 2, lines 14-16; Column 3, lines 32-49). The alloantigen is indirectly attached to a solid support, contacted with a biological sample, and washed; the presence of bound alloantigen-specific receptor is then detected with a labeled reagent. The '841 patent does not teach, disclose or even suggest a method that utilizes soluble MHC trimolecular complexes, and most certainly does not teach that said soluble MHC trimolecular complexes are produced in a mammalian cell line that expresses multiple surface-bound native Class I endogenous MHC molecules and that are subsequently purified substantially away from other proteins.

The Examiner has recognized the deficiencies of the '841 patent, and has attempted to supply the same with **FOUR** secondary references.

The '641 patent adds nothing to supply the defect of the '841 patent; that is, the '641 patent also does not teach, disclose or even suggest a method that utilizes soluble MHC trimolecular complexes, and most certainly does not teach that said soluble MHC trimolecular complexes are produced in a mammalian cell line that expresses multiple surface-bound native Class I endogenous MHC molecules and are then purified substantially away from other proteins. Therefore, the **combination** of the '841 and '641 patents do not teach, disclose or even suggest the presently claimed invention.

While it is agreed that Prilliman et al. teaches production of soluble HLA molecules, Prilliman et al. specifically teach the use of a **Class-I deficient HLA cell line** (see Page 380, Column 2, lines 22-25). Therefore, Applicants respectfully submit that Prilliman et al. does not teach, disclose or even suggest producing soluble MHC trimolecular complexes in a mammalian cell line that expresses multiple surface-bound native Class I endogenous MHC molecules. Moreover, a person having ordinary skill in the art would be aware that **all MHC/HLA purification methods prior to the present invention utilized MHC/HLA-deficient cell lines**, so that multiple, endogenously produced MHC/HLA did not interfere with the purification

methods. In fact, prior to the present invention, it was very difficult if not impossible to separate recombinantly produced MHC from endogenously produced MHC when both were produced by a single cell. **Therefore, the presently claimed invention is nonobvious over the prior art in that it produces soluble HLA molecules in ANY cell line, and does not require that the cell line be HLA-deficient.**

In addition, the Prilliman et al. reference is directed to large-scale production of class I bound ***peptides***. Prilliman et al. teach the production of soluble HLA-B*1501 molecules that are passed over a column of light-chain specific antibody coupled to CNBr-activated Sepharose 4B. However, **the trimolecular complexes are not eluted off the column**; rather, the column is subjected to ***acid***, which ***denatures* the trimolecular complexes** and elutes the three components thereof (i.e., the heavy chain molecule, beta-2-microglobulin and peptide) separately.

Submitted herewith are unequivocal declarations by Dr. William Hildebrand, Ph.D., and Dr. Rico Buchli, Ph.D., who are co-inventors of the subject application. Dr. Hildebrand is also a co-author of the Prilliman et al. reference. Such declarations clearly state that the Prilliman et al. paper does not provide functionally active, individual soluble MHC trimolecular complexes that are purified substantially away from other proteins, but rather provides ***denatured complexes*** from which peptides can be obtained. Attached to the declaration by Dr. Buchli is Exhibit B, which contains experimental data that clearly demonstrates this fact.

Therefore, Prilliman et al. do not teach a **functionally active**, individual soluble MHC **trimolecular complex that is purified substantially away from other proteins** such that the individual soluble MHC trimolecular complex maintains the physical, functional and antigenic integrity of the native MHC trimolecular complex.

Therefore, the **combination** of the '841 and '641 patents and Prilliman et al. do not teach, disclose or even suggest the presently claimed invention.

DiBrino et al. teach production of full-length HLA in a **Class I-deficient HLA cell line**, and therefore do not supply the deficiencies of the other prior art references. Thus, the **combination** of the '841 and '641 patents, Prilliman et al., and DiBrino et al. do not teach, disclose or even suggest the presently claimed invention.

The Examiner's assertion that **her own reference** teaches the use of a "class I deficient cell line", followed by the application of a reference that teaches that said cell line *actually expresses* class I HLA molecules, is not well taken. However, in response to the Examiner's application of the Zemour et al. reference, Applicants respectfully submit the following: (1) Zemmour et al. teach a cell line that is negative for **native** HLA-A,B expression; (2) while Zemmour et al. teach expression of HLA-C proteins in their cell line, this expression level is a fraction of the expression of HLA-A,B molecules; and (3) while Zemmour et al. teach expression of an HLA-B35 allele, said protein is a **novel, non-native subtype**, and is produced at greatly reduced levels that are not even detectable by antibodies or complement. See, for example, the following citations from Zemmour et al.:

> In general, cell surface expression of HLA-C proteins is about **one-tenth** that of HLA-A,B molecules, and this ratio is maintained when HLA-A or B genes are transfected into C1R. As a consequence, the expression of Cw4 by C1R has not interfered significantly with analysis of normal or mutant HLA-A,B genes transfected into C1R. (Page 1941, Column 2, lines 16-20)

> Transcription of the HLA-B35 gene is also normal and comparable to that of HLA-Cw4. **However, expression of the HLA-B35 protein is reduced to a few percent of the parental level.** Comparison of the nucleotide sequence of B35 alleles from C1R and Hmy2 revealed that reduced translation in C1R is caused by a point mutation (ATG to TTG) in the translation initiation codon. The HLA-B35 allele from C1R and Hmy2 represents a **novel subtype, B*3503, differing from B*3501 by replacement of serine by phenylalanine at the peptide binding position 116.** This study shows cell surface levels of a class I molecule which are insensitive to lysis by antibody and complement... (Abstract, Page 1941, Column 1, lines 19-32)

Therefore, Zemmour et al. do not teach, disclose or even suggest a mammalian cell line that meets the claim limitation of expressing "***multiple*** surface-bound ***native*** Class I endogenous MHC molecules", and thus cannot be relied upon to remedy the deficiencies of the other four cited references.

Therefore, the *combination* of the '841 and '641 patents, Prilliman et al., DiBrino et al., and Zemmour et al. do not teach, disclose or even suggest the presently claimed invention.

Thus, Applicants respectfully submit that the Examiner cannot possibly assert that the references were argued separately, as Applicants have clearly responded to the rejection of the **combination** of references as a whole and have not attacked references individually. Therefore, Applicants respectfully submit that the Examiner cannot apply *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); and *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986) to rebut this response to the 35 U.S.C. 103(a) rejection.

Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. 103(a) rejection of claims 31-37, 42, 45, 46, 48-51, 60 and 61 over US 5,482,841 in view of US 5,292,641, Prilliman et al., DiBrino et al., and Zemmour et al.

Applicants' Response to the Second 35 U.S.C. 103(a) Rejection

In the Office Action, the Examiner rejected Applicants' claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 103(a) as being unpatentable over US 5,482,841 in view of US 5,292,641, US 2002/0197672, Prilliman et al., and DiBrino et al. Applicants respectfully traverse the rejection for the reasons stated herein below.

Applicants respectfully incorporate herein all remarks presented herein above in response to the first 35 U.S.C. 103(a) rejection that were related to the combination of the '841 and '641 patents and the Prilliman et al. and DiBrino et al. references. In summary, Applicants respectfully submit that the combination of said references do not teach, disclose or even suggest a method for detecting the presence of anti-MHC antibodies in a sample that utilizes a pool of functionally active, recombinantly produced, truncated individual soluble MHC trimolecular complexes that are directly or indirectly linked to a substrate, wherein the MHC trimolecular complexes are produced in a mammalian cell line that **expresses multiple surface-bound native Class I endogenous MHC molecules**, and wherein the MHC trimolecular complexes are **purified substantially away from other proteins** prior to linkage to a substrate.

US 2002/0197672 is a publication of co-inventor William Hildebrand's earlier patent application, US Serial No. 09/974,366. The application is related to "a methodology for the isolation, purification and identification of **peptide ligands** presented by MHC positive cells" (Abstract). Said publication specifically states that "peptide ligands were purified from class I

molecules by acid elution (Prilliman, KR et al., Immunogenetics 48:89, 1998 which is expressly incorporated herein by reference)" (see paragraph [0104]). Thus, based on the Declarations of co-inventors William Hildebrand and Rico Buchli, it is clearly apparent that US 2002/0197672 simply provides teachings that are cumulative to the Prilliman et al. reference; thus, US 2002/0197672 is no more pertinent than Prilliman et al., and therefore does not remedy the deficiencies of the combination of the '841 and '641 patents and the Prilliman et al. and DiBrino et al. references.

Therefore, Applicants respectfully submit that claims 31-37, 42, 45, 46, 48-51, 60 and 61 are non-obvious over US 5,482,841 in view of US 5,292,641, US 2002/0197672, Prilliman et al., and DiBrino et al. Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. 103(a) rejection thereof.

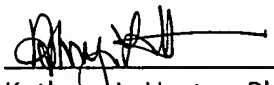
CONCLUSION

This was meant to be a complete response to the Office Action mailed July 22, 2008. Applicants respectfully submit that each and every rejection of the claims has been overcome. Further, Applicants respectfully submit that pending claims 31-37, 42, 45-46, 48-51 and 60-61, as now amended, are free of the prior art of record and are in a condition for allowance. Favorable action is respectfully solicited.

In addition, claims 38-41 are currently withdrawn; however, upon allowance of any of claims 31-37, 42, 45-46, 48-51 and 60-61, Applicants respectfully request rejoinder and reconsideration of currently withdrawn claims 38-41. In addition, the Examiner previously required election of a single disclosed species to be used in the claimed method (i.e., a specific substrate, soluble HLA molecule, antibody and anchoring moiety). Upon allowance of any of claims 31-37, 42, 45-46, 48-51 and 60-61, Applicants respectfully request rejoinder and reconsideration of all disclosed and claimed species (i.e., all specific substrates, soluble HLA molecules, antibodies and anchoring moieties).

Should the Examiner have any questions regarding this amendment, or the remarks contained therein, Applicants' representative would welcome the opportunity to discuss same with the Examiner.

Respectfully submitted,



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